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DATE: **April 10, 2001**

SUBJECT: ***DISULFOTON:*** **3rd Report** of the Hazard Identification Assessment Review Committee

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THRU: Jess Rowland, Chair
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PC Code: 032501

On December 19, 2000 and January 10, 2001 the Health Effects Division's Hazard Identification Assessment Review Committee evaluated the toxicity data used for endpoints in short-term occupational/residential exposure. The Committee did not address other endpoints. The Committee's conclusions are presented in this report.

Members in Attendance

Hazard Identification Assessment Review Committee members in attendance: William Burnam, , Beth Doyle, Pamela Hurley, Elizabeth Mendez, Ayaad Assaad, Yung Yang, Jonathan Chen, David Nixon, Jess Rowland and Brenda Tarplee (Executive Secretary).

Data Presentation:	_____
and	David G Anderson
Report Preparation	Toxicologist

1. INTRODUCTION

On **April 25, 1996**, the Health Effects Division's RfD/Peer Review Committee evaluated the toxicology data base of Disulfoton and established the Reference Dose (RfD) of 0.0003 mg/kg/day based on a NOAEL of 0.025mg/kg/day and an Uncertainty Factor of 100 for inter species extrapolation and intraspecies variation *(Memorandum*: G.Ghali to G. LaRoca, April 21, 1997).

On **May 14, 1996,** the Toxicology Endpoint Selection Committee selected the doses and endpoints for acute dietary and occupational as well as residential exposure risk assessments (TES Document 6/5/96).

On **November 20, 1997**, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) re-evaluated the toxicology data base, re-assessed the RfD and selected the toxicology endpoints for acute dietary as well as occupational and residential exposure risk assessments. In addition, the HIARC also addressed the potential enhanced susceptibility of infants and children from exposure to disulfoton as required by the Food Quality Protection Act (FQPA) of 1996.

On **April 9, 1998**, the HIARC reviewed the results of a two-generation reproduction study in rats (MRID# 44440801) that was recently submitted to the Agency and the impact of this study in the doses and endpoints selected for the various risk assessments.

On **May 12-14, 1998**, the HIARC conducted a comprehensive review of 40 organophosphates, including disulfoton. At this meeting it was concluded that the toxicology database is inadequate since there was a data gap for an acceptable acute delayed neurotoxicity study in the hen. Subsequently, the requirement of a developmental neurotoxicity study was 'reserved' at this time.

On **January 19, 2000**, the HIARC reviewed the results of a new acute delayed neurotoxicity study in the hen. In addition, the equivocal results of a 90-day neurotoxicity study in rats were reviewed for potential disulfoton induced neuropathy. HIARC also evaluated the toxicology data base for disulfoton to determine whether a DNT was triggered None of the endpoints for any of the RfDs or occupational or residential exposure were changed from the previous HIARC.

On **December 19, 2000 and January 11, 2001**, the HIARC reviewed the results from a 3-day dermal toxicity study in rats (recently submitted to the Agency) in conjunction with the available 21-day dermal toxicity studies in rabbits. These studies were evaluated together to determine their suitability for use in short-term dermal risk assessment.

The report supercedes the previous HIARC reports

2. HAZARD IDENTIFICATION

2.1. Acute Reference Dose (RfD)

Study Selected: Acute Neurotoxicity - Rat §81-8

MRID No. 42755801

Executive Summary: In an acute neurotoxicity screening study, disulfoton (97.8% a.i.) was administered in a single gavage dose to 10 male Sprague-Dawley rats at doses of 0, 0.25, 1.5, or 5.0 mg/kg and to 10 female Sprague-Dawley rats at doses of 0, 0.25, 0.75 or 1.5 mg/kg. These rats were assessed for reactions in functional observational battery (FOB) and motor activity measurements at approximately 90 minutes post-dosing and on days 7 and 14. Cholinesterase determinations (erythrocyte and plasma) were made at 24 hours post-dosing. Six rats/sex/dose were examined for neuropathological lesions.

At 0.75 mg/kg, 4/10 females had muscle fasciculations. At 1.5 mg/kg, males had muscle fasciculations, diarrhea, and sluggishness and females also had tremors, ataxia, oral staining, decreased activity/sluggishness, decreases in motor and locomotor activity (38-49% of control), and a slightly increased duration of nasal staining. One female at 1.5 mg/kg died from cholinergic intoxication on the day of dosing. At 5.0 mg/kg, males also had symptoms similar to those observed in females at 1.5 mg/kg/day, including reduced motor/locomotor activity (36-45% of control). Recovery appeared to be complete in surviving animals by Day 14. Based on the evidence of neurotoxicity (probably associated with inhibition of cholinesterase) in females at 0.75 mg/kg, the study LOAEL is 0.75 mg/kg and the study NOAEL is 0.25 mg/kg.

At 0.75 mg/kg in females, cholinesterase activities were inhibited by 53% (erythrocyte) and 30% (plasma) and by 75% (erythrocyte) and 52% (plasma) at 1.5 mg/kg in females. At 5.0 mg/kg in males, cholinesterase activities were inhibited by 21% (erythrocyte) and 25% (plasma). The LOAEL for inhibition of cholinesterase activity is 0.75 mg/kg and the NOAEL for inhibition of cholinesterase activity is 0.25 mg/kg.

Dose and Endpoints for Establishing the Acute RfD: NOAEL= 0.25 mg/kg based on neurotoxicity signs, plasma and erythrocyte cholinesterase inhibition in female rats.

Uncertainty Factor (UF): 100

Comments about the study and/or Endpoint: This dose and endpoint is appropriate since the toxicological effects were observed following a single oral dose.

$$\textbf{Acute RfD} = \frac{\textbf{0.25 mg/kg (NOAEL)}}{\textbf{100 (UF)}} = \textbf{0.0025 mg/kg}$$

2.2 Chronic Reference Dose

Study Selected: Chronic Feeding Dog §83-1

MRID No. 44248002

Executive Summary: In a chronic toxicity study, disulfoton (97% a.i.) was administered orally in the diet to purebred beagle dogs (4/sex/dose) at dose levels of 0.5, 4 or 12 ppm (equivalent to 0.015, 0.121 and 0.321 mg/kg/day for males; and 0.013, 0.094 and 0.283 mg/kg/day for females) for one year. Potential ocular and neurologic effects were addressed. Plasma cholinesterase was decreased starting at day 7 in the 4.0 ppm dose groups of the study through to termination (males 39% to 46%; females 32% to 45%). Erythrocyte cholinesterase was decreased starting at day 91 in the 4.0 ppm dose groups through to termination (males 23% to 48%; females 17% to 49%). Not all the values at 4.0 ppm were statistically significant, probably because of the wide range in values, but at least 2 animals per group showed biologically significant cholinesterase inhibition. By termination cholinergic effects of the plasma, erythrocytes, brain, and ocular tissues were observed in both sexes in the 4 and 12 ppm treatment groups. Plasma and erythrocyte cholinesterase depression are compared to pretreatment values. Brain, cornea, retina and ciliary body cholinesterase depression are compared with concurrent control values at termination only. In the 12 ppm treatment groups, depressed cholinesterase was observed in plasma (56%-63%), erythrocytes (30%-91%), and brain (32%-33%) compared to their respective controls. In the 4 ppm treatment groups in males and females, cholinesterase was depressed in plasma (38%-46%), erythrocytes (40%- 38%), and brain (females only, 22%). Disulfoton inhibited cholinesterase of the cornea, retina, and ciliary body, but did not appear to alter the physiologic function of the visual system. In the 12 ppm treatment groups, depressed cholinesterase was observed in the cornea (60-67%), ciliary body (45-54%), and retina (males only; 67%). In the 4 ppm treatment groups, cholinesterase was inhibited in the cornea (50-60% lower), and retina (females only, 25%). No treatment-related ophthalmology findings or histological or electrophysiological changes in the retina were observed. No other treatment-related effects were observed. No animals died during the study. No treatment-related effects were observed in systemic toxicity including food consumption, body weights, clinical signs, hematology, clinical blood chemistry or urinalysis parameters, electrocardiogram, electroretinograms or clinical neurological findings, organ weights or gross or microscopic post-mortem changes in any treatment group. No neoplastic tissue was observed in dogs in the treatment and control groups. The LOAEL is 4 ppm (0.094 mg/kg/day), based on depressed plasma, erythrocyte, and corneal cholinesterase levels in both sexes, and depressed brain and retinal cholinesterase levels in females. The NOAEL is 0.5 ppm (0.013 mg/kg/day). These LOAEL/NOAEL for plasma cholinesterase inhibition extend from day 7 to termination and for erythrocyte cholinesterase inhibition they extend from day 91 to termination.

Dose and Endpoint for Establishing the Chronic RfD: The NOAEL is 0.5 ppm (0.013 mg/kg/day) based on depressed plasma, erythrocyte and corneal cholinesterase levels in both sexes and depressed brain and retinal cholinesterase levels in females.

Uncertainty Factors (UF): 100

Chronic RfD = $\frac{\text{0.013 mg/kg (NOAEL)}}{\text{100 (UF)}}$ = 0.00013 mg/kg/day

2.3. Occupational/Residential Exposure

2.3.1. Dermal Absorption; § 85-2

MRID No.: 43360201

The test material was applied to the backs of rats at 0.85, 8.5, and 85 $\mu g/cm^2$ (approximately 0.051, 0.51 and 5.1 mg/kg). The percent of absorbed dose at 10 hours post-application was 26, 36, and 25%, respectively.

Dermal Absorption Factor: 36%

Comments about the Study Endpoint: The HIARC indicated that dermal absorption of 36%, obtained after 10 hours exposure at a concentration of 8.5 $\mu g/cm^2$ (0.51 mg/kg), should be used for correcting oral dosing to dermal dosing. The HIARC concurred with the TES Committee on this approach for the use of the dermal absorption factor. HIARC deviated from the standard practice of using the 10-hour dermal absorption value from the lowest application rate in this case because of the lack of a coherent pattern of absorption normally observed in dermal absorption studies. In most cases, the lowest application rate results in the highest dermal absorption rate, with declining absorption at higher applications. This is assumed to reflect overloading of the site of application. In as much as there was no dose-related pattern to the percent of disulfoton absorbed, HIARC elected to use the 36% absorption rate to reduce the likelihood of underestimation.

2.3.2 Short Term Dermal - (1-7 DAYS)

Study Selected: 3-day dermal study in rats

MRID No. 45239602

Executive Summary: In a 3-day dermal rat study (MRID# 45239602) disulfoton, granular, 1% a.i. (1% G Di-Syston®) was administered dermally to 5 Wistar (Crl:WI(HAN)BR) rats/sex/dose at 0, 50, 100, 200 or 500 mg/kg/day (equivalent to 0, 0.5, 1.0, 2.0 or 5.0 mg a.i./kg/day). Plasma and erythrocyte cholinesterase was measured at 24 hours after the first and day 3 dose. Brain cholinesterase was measured at termination on day 4. Test material was ground and applied to plastic backed gauze, moistened with water, applied to the shaved test site (about 10% of the body surface), and then secured with a bandage. The animals were exposed dermally for 6 hour per day with washing at the end of the exposure period.

No clinical signs were noted or body weight decrement. No other signs of toxicity were noted, but the study was designed to determine cholinesterase depression only. After 1 day of dosing, the NOAEL in males was 200 mg/kg and the LOAEL was 500 mg/kg based on biologically significant 31% erythrocyte cholinesterase inhibition which was not statistically significant.

After 1 day of dosing the NOAEL in females was 100 mg/kg and the LOAEL was 200 mg/kg based on biologically significantly increased inhibition of plasma cholinesterase (36%). After 3-days of dermal dosing the NOAEL in males was 100 mg/kg/day and LOAEL was 200 mg/kg/day based on a increase in brain cholinesterase inhibition of 21% (statistically significant). After 3 days of dosing the NOAEL in females was 50 mg/kg/day and the LOAEL was 100 mg/kg/day based on statistically significant plasma and brain cholinesterase inhibition of 37% and 18%, respectively.

The overall NOAEL of 100 mg/kg/day (equivalent to 1.0 mg a.i./kg/day) with a LOAEL of 200 mg/kg/day (equivalent to 2.0 mg a.i./kg/day) was based on female plasma cholinesterase depression for 1 day of dosing. After 3 days of dosing the NOAEL was 50 mg/kg/day (equivalent to 0.50 mg a.i./kg/day) with a LOAEL of 100 mg/kg/day (equivalent to 1.0 mg a.i./kg/day) based on depressed plasma and brain cholinesterase in females.

The study is acceptable for selecting a regulatory endpoint although neither a 1-day nor a 3-day dermal study in the rat is a guideline study.

Dose and Endpoint for risk assessment: The NOAEL = 0.5 mg/kg/day based on plasma and brain cholinesterase inhibition in females rats at 1.0 mg/kg/day (LOAEL).

Comments about the study and/or endpoint: The 3-day dermal study was the most appropriate for several reasons. It was conducted in the appropriate species. Cholinesterase was seen in two compartments, plasma and brain. The LOAEL of 1.0 mg/kg/day in the selected study is supported by the LOAELs in the 1988 and 1986 21-day dermal studies in the rabbit of 1.0 and 1.6 mg/kg/day, respectively at day 8 to 15. The test material consisted of the granular material to which the handlers are exposed.

A 21-day dermal toxicity study in rabbits (1988) with a NOAEL = 0.8 mg/kg/day and a LOAEL = 1.0 mg/kg/day (based upon plasma cholinesterase inhibition at Day 8) was not selected for this endpoint. The NOAEL for this study overlapped the LOAEL in the developmental toxicity study in rats, suggesting that the rat is more sensitive than the rabbit to the effects of disulfoton. Therefore, the HIARC selected the NOAEL from the 3-day dermal study in rats. In addition, the NOAEL of 0.5 mg/kg/day was selected in preference to the 0.3 and 0.4 mg/kg/day NOAELs from the developmental toxicity study in rats and the 21-day dermal study in rabbits (1986) because the LOAELs from all three studies were similar and the spread between 0.3 and 0.5 mg/kg/day is likely due to dose selection and does not reflect differential toxicity.

2.3.3 Intermediate Term Dermal Exposure (1 Week to Several Months):

Study Selected - Special 6-months cholinesterase study

MRID No.: 43058401

Executive Summary: In a 6-month study designed to establish a NOAEL and LOAEL for cholinesterase inhibition, technical grade disulfoton (98-99% a.i.) was administered in the diet to 35 male and female Fisher 344 rats for up to 6 months at levels of 0, 0.25, 0.5 or 1 ppm (approximate doses of 0, 0.02, 0.03 or 0.06 mg/kg/day for males and 0, 0.02, 0.03 or 0.07

mg/kg/day for females). At the end of 2, 4 and 6 months, 10 rats/sex/dose were taken for blood and brain cholinesterase assays. Statistically significant inhibition of cholinesterase activity was observed in erythrocytes in females at all doses (3-14% inhibition, 11-17% inhibition, and 23-29% inhibition at 0.24, 0.5, and 1.0 ppm, respectively. In addition, at 1.0 ppm, males had decreased erythrocyte cholinesterase activity (10-16% inhibition) and females had decreased plasma (8-17% inhibition) and brain (7-13% inhibition) cholinesterase activities. However, biologically significant and statistically significant inhibition of cholinesterase activity was observed only in the plasma, erythrocytes and brain of females at 1.0 ppm. No biologically significant inhibition of cholinesterase activity was observed in males. The LOAEL for inhibition of cholinesterase activity was 1.0 ppm is based on a 23-29% inhibition of erythrocyte, 12-17% inhibition of plasma and 13% inhibition of brain cholinesterase in females. The NOAEL is 0.5 ppm (0.03 mg/kg/day). No biological meaningful cholinesterase inhibition was observed in males at any dose level. Body weight, food consumption, and clinical signs were also monitored, but showed no treatment related effects. Based on these few parameters, no systemic effects were observed at any dose level and the NOAEL for systemic toxicity was 1.0 ppm (0.06 mg/kg/day for males and 0.07 mg/kg/day for females).

Dose and Endpoint for use in risk assessment: NOAEL=0.03 mg/kg/day was based on plasma, erythrocyte and brain cholinesterase inhibition in female rats at 0.07 mg/kg/day (LOAEL).

Comments about study and/or endpoint: Since an oral NOAEL was identified, a dermal absorption factor of 36% should be used for this risk assessment. This endpoint is supported by similar effects (plasma, erythrocyte and brain cholinesterase inhibition) observed in a subchronic neurotoxicity study in rats (MRID# 42977401). A comparison of the oral developmental studies with the oral 90-day neurotoxicity study (MRID# 42977401) shows a relationship between increased cholinesterase inhibition with increased duration of the study. The dermal equivalent of LOAEL of 0.2 mg/kg/day in the 90-day study (i.e., 0.07 mg/kg/day ÷ 0.36 = 0.2 mg/kg/day) at 4 weeks is lower than the LOAEL from either the 1986 (LOAEL=1.6 mg/kg/day) or the 1988 21-day rabbit dermal studies (LOAEL=1.0 mg/kg/day) at the end of 3 weeks. These comparisons support the generally held perception that rabbit dermal studies tend to underestimate toxicity from an organo-thiophosphate pesticide. The new 2-generation study on reproduction (MRID# 44440801) also supports the 6-month cholinesterase study endpoints.

2.3.4. Long-Term Dermal (Several Months to Life Time)

Study selected: Chronic Toxicity -Dog §83-1

MRID No. 44248002

Executive Summary: See summary under Chronic RfD.

Dose and Endpoint for Risk Assessment: NOAEL=0.013 mg/kg/day based on depressed plasma, erythrocyte and corneal cholinesterase levels in both sexes and depressed brain and retinal cholinesterase levels in females.

Comments about study and/or endpoint: This dose was used to establish the chronic RfD. Since an oral NOAEL was identified, a dermal absorption factor of 36% should be used for this

risk assessment.

2.3.5. Inhalation Exposure (All Time Periods)

Study Selected: 90-Day Inhalation - Rat §82-4

MRID No.: 41224301

Executive Summary: Disulfoton was administered by inhalation to 12 Fisher 344 rats per sex per group for air control, polyethylene glycol-400: 50% ethanol vehicle control, 0.015, 0.15 or 1.5 mg/m^3 nominal dose levels for 90-days in a nose only chamber. The analytical determined mean dose levels were 0, 0, 0.018, 0.16 and 1.4 mg/m^3 for male and female rats. The rats were exposed to the test material 6 hours per day, 5 days per week. The particle sizes in the inhalation chambers had a MMAD ± geometric standard deviation of 1.3 ± 1.4, 1.1 ± 1.3, 1.0 ± 1.3 and 1.1 ± 1.4 μm for the two controls, 0.015, 0.15 and 1.5 mg/m^3 nominal dose levels, respectively. The range in mean daily particle sizes had a MMAD of 0.5 ± 1.0 μm to 2.6 ± 1.6 μm. At the highest dose level, plasma cholinesterase was depressed in males (19% and 14% from air controls at 38 days and term, respectively, $p \leq 0.05$) and in females (27% and 31% from air controls at 38 days and term, respectively, $p \leq 0.05$). Brain cholinesterase was depressed in males (29%) and females (28%) at termination. Erythrocyte cholinesterase was depressed in females at 38 days (11% at 38 days, $p \leq 0.05$, not considered biologically relevant) at 0.16 mg/m^3 and higher in males and females at 1.4 mg/m^3 at 38 days and term. Brain cholinesterase was depressed (10%, $p \leq 0.05$) at 0.16 mg/m^3, but this degree of variation was not considered biologically relevant due to variation noted in this parameter. Inflammation of the male nasal turbinates occurred at 1.4 mg/m^3. No other test material related effects were noted. The NOAEL/LOAEL is 0.16/1.4 mg/m^3 for plasma, erythrocyte and brain cholinesterase depression in males and/or females.

Dose and Endpoint for use in risk assessment: NOAEL=0.00016 mg/L based on plasma, erythrocyte and brain cholinesterase inhibition. The rat inhalation NOAEL when converted to mg/kg is 0.045 mg/kg/day.

Conversion [1] of mg/L to mg/kg/day using route-to-route extrapolation policy. [0.00016 mg/L (NOAEL) x 1(fractional absorption)x 7.15 L/hr (respiratory volume for Fisher 344 rats)x 6(hours)x 1(activity factor)]/[0.152 kg (body weight)] = 0.045 mg/kg.

Comments about the study and/or endpoint: This NOAEL will be used for inhalation exposure risk assessments for any time period (i.e., Short, Intermediate and Long-term). An inhalation toxicity study with 3 to 5 day exposure was available. In that study, the LOAEL was <0.0005 mg/L (lowest dose tested); a NOAEL was not established. Although this study could have been used for the Short-Term exposure risk assessment, the HIARC did not use this study because: (i) it demonstrated a LOAEL rather than a NOAEL; (ii) the use of a LOAEL would have required an additional 3 x UF; and (iii) the value derived from the use of the LOAEL and 3 UF (0.0005 ÷ 3= 0.00017 mg/L) is comparable to the NOAEL of 0.00016 mg/L in the 90-day study.

[1] Memorandum of 10/10/98 from John Whalan to Margaret Stasikowski, HED. Route-to-Route Extrapolation, page 8.

2.3.6. Margins of Exposure for (Occupational/Residential) Exposures

A Margin of Exposure (MOE) of 100 is adequate for occupational exposure risk assessments. The FQPA Safety Committee determined that an MOE of 100 is adequate for residential exposure risk assessments.

2.4. Recommendation for Aggregate Exposure Risk Assessment

For acute, short, intermediate and long-term de aggregate exposure risk assessment, the oral, dermal and inhalation routes can be combined since a common toxicological endpoint (cholinesterase) was observed during all routes of exposure (oral, dermal and inhalation) in the toxicity studies.

3. CLASSIFICATION OF CANCER POTENTIAL

The HED RfD/Peer Review classified disulfoton as a Group E Chemical-Not Classifiable to Carcinogenicity based on the lack of evidence of carcinogenicity study in mice and rats at dose levels adequate to test for carcinogenicity.

4. MUTAGENICITY

The following was taken from a document written by Nancy McCarroll for the Hazard Identification Assessment Review Committee proceedings. Combining the acceptable studies with the additional EPA-sponsored studies will satisfy the Pre-1991 mutagenicity initial testing battery guidelines. No further mutagenicity testing has been identified at this time. In addition, disulfoton is not genotoxic *in vivo* or carcinogenic in mice or rats.

In some of the mutagenicity studies, positive effects were seen without activation while negative effects were seen with activation. This may be due to microsomal enzyme metabolism, since pretreatment of rats and mice with phenobarbital reduces toxicity from disulfoton.

4.1 Gene Mutation (84-2)

Salmonella typhimurium/Escherichia coli reverse gene mutation plate incorporation assay (Accession No. 00028625; Doc. No. 003958: As part of an Agency sponsored mutagenicity screening battery, disulfoton was negative in all strains up to the HTD (5000 μg/plate +/- S9) in three independent trials.

Chinese hamster ovary (CHO) cell HGPRT forward gene mutation assay (MRID# 40638401, Doc# 008394): This unacceptable study is considered to be positive, because the assay was conducted at partially soluble levels(0.1-1.0 μL/ml -S9; 0.7-1.0 μL/ml +S9) and insoluble doses (5-10 μL/ml -S9; 3-10 μL/ml +S9) but not active at soluble concentrations ($\leq$0.06 μL/ml +/-S9). The mutagenic response appeared to be stronger without metabolic (S9) activation .

4.2 Chromosome Aberrations (84-2)

Mouse micronucleus test (MRID No. 43615701) No increase over background in micronucleated

polychromatic erythrocytes (evidence of cytogenetic damage) of mice treated intra-peritoneally up to MTD levels (8 mg/kg). Lethality and other signs of toxicity, but no bone marrow cytotoxicity was seen.

4.3 Other Gene Mutations: (84-2)

Bacterial DNA Damage/Repair: *E. Coli* DNA damage/repair test (Accession# 072293; Doc# 004698): The test is negative up to the HDT (10,000 μg/plate +/- S9.

Mitotic Recombination: *Saccharomyces cerevisiae* D3 mitotic recombination assay (Accession# 00028625; Doc# 003958): Disulfoton (up to 5% +/- S9) was negative at this endpoint in the Agency-sponsored mutagenicity screening battery. The study is currently listed as unacceptable, but should be upgraded to acceptable. Upon further review of the data, it was decided that the reason for rejecting the study (number of replicates/dose not provided) did not interfere with the interpretation of the findings.

Sister Chromatid Exchange: Sister chromatid exchange in CHO cells (MRID# 4095001; Doc# 008394): Positive, dose related effects at 0.013-0.1 μL/ml without S9, but not active in the S9 activated phase of testing up to a level (0.20 μL/ml) causing cell cycle delay.

Sister Chromatid Exchange: Sister chromatid exchange in Chinese hamster V79 cells (Accession# 072293; Doc# 0044223): The test is negative without activation up to the HTD (80 μg/ml). Subsequently tested by the same investigators (Chen et al., 1982; Environ. Mutagen. 4: 621-624) in the presence of exogenous metabolic activation and found to be negative up to the HDT (80 μg/ml).

Unscheduled DNA Synthesis (UDS): UDS in WI-38 human fibroblasts (Accession# 000028625; Doc# 003958): The test is positive in the absence of S9 activation at precipitating doses (1000-4000 μg/ml). With S9 activation, the study was negative at comparable percipitating concentrations.

4.4 Other EPA Sponsored Studies:

Disulfoton was also included in second tier mutagenicity test battery performed at the EPA (EPA-600/1-84-003) in 1984. Although DERs have not been prepared for these additional assays, we assess that they are acceptable for regulatory purposes.

Mouse Lymphoma L5178Y TK+/- forward gene mutation assay: The test was positive in the absence of S9 activation with concentration dependent and reproducible increases in mutation frequency at 40-90 μg/ml; higher dose levels were cytotoxic. No mutagenic activity was seen in the presence of S9 activation up to a cytotoxic dose (150 μg/ml).

Mouse Micronucleus Assay: The test is negative in Swiss Webster mice up to a lethal dose (8 mg/kg) administered once daily for 2 consecutive days by intra-peritoneal injection. No bone marrow cytotoxicity was seen.

Sister Chromatid Exchange in CHO cell assay: The non-activated test was negative up to levels ($\geq$0.02%) that caused cell cycle delay, but the test material was weakly positive at a single dose (0.04%) with metabolic activation.

5. FQPA CONSIDERATIONS

5.1. Adequacy of the Data Base

The toxicity data base is adequate to determine the neurotoxic potential from disulfoton exposure, except for developmental neurotoxic potential. A developmental neurotoxicity study for organophosphates, including disulfoton is required.

5.2. Neurotoxicity

Another acute delayed neurotoxicity study (81-7) was submitted and reviewed and is acceptable. The study is negative for organophosphate induced delayed neuropathy (OPIDN). Absolute brain weight was not affected by treatment in the guideline chronic studies in rodents. (The subchronic studies, which were graded unacceptable, were not provided for review.) In the rat study, treatment-related eye lesions were seen (optic nerve degeneration and corneal vascularization) and skeletal muscle atrophy were observed. The optic nerve degeneration was related to orbital sinus bleeding injury, so results were not considered treatment related. These neuropathological findings were not repeated in the 1997 1-year dog study, but cholinesterase levels in the cornea, retina, and ciliary body were depressed with treatment. No treatment related neuropathy was seen in acute or in 90-day neurotoxicity studies in rats. The marginal elevation in lesions seen the optical nerve and thoracic spinal cord at the highest dose tested were not considered to be sufficiently different from control lesions to indicate a treatment-related effect had occurred.

The repeat "acute delayed neurotoxicity study in hens" requested by the HIARC of April 23, 1998 is summarized below.

In an acute delayed neurotoxicity study in hens (MRID# 44996401, 1999), disulfoton was acutely administered orally to 18 LSL laying hens at 40 mg/kg bird in a single dose. Fifteen hens were used as controls. Doses were administered in aqueous 2% Cremophor at 5 ml/kg bird. Five to 18 minutes

before administration of the disulfoton, atropine was administered s.c. (0.5 ml/kg of 4% atropine sulfate). Directly prior to the administration of the disulfoton, 0.5 ml/kg of 10% atropine sulfate and 10% 2-PAM chloride was injected s.c. The afternoon of day 0, 0.5 ml/kg of 5% atropine sulfate and 5% 2-PAM chloride was injected s.c. and again the morning and afternoon of day 1. Clinical observations were made at least daily. Forced motor activity tests were conducted by forcing the hens to run around a 12-13 m^2 area and rated for coordination, ataxia, and paresis. NTE studies were conducted at 24 and 48 hours on the spinal cords, sciatic nerves and ½ of the brain in each of 3 hens per group. Cholinesterase activity studies were conducted on the other ½ of the brain from each bird in the NTE study at 24 and 48 hours post treatment. The study was conducted at 1.4 times the LD50 for hens.

No typical signs of organophosphate induced delayed neuropathy was seen during the study or on microscopic examination of the treated birds at termination at 3 weeks. No inhibition was seen in the NTE study at 24 hours or 48 hours. Inhibition was low between 4% and 8% and was not considered to be indicative of OPIDN. Cholinesterase activity in the brain was inhibited 83% and 59% at 24 and 48 hours, respectively.

No hens died, but by day 7 there was a decrease in body weight of over 5%. The hens slowly recovered and by the end of 3 weeks, body weight of the treatment group and of the controls did not differ.

Severely uncoordinated gait was observed in all treated birds within 5 minutes of being dosed with atropine and before disulfoton treatment. The report authors attributed this abnormal gait to atropine since it lasted only for the duration of the atropine treatment (2 days). However, the report authors also noted reduced motility in 1-3 birds for 0-1 day, which they attributed to disulfoton treatment. Neither statements are completely supportable because the hens were dosed with atropine and disulfoton during most of this period. However, the temporary uncoordinated gait was followed by no microscopic findings in nerve tissue and no other signs, which supports a conclusion of no demonstrated OPIDN in hens dosed with disulfoton.

Microscopic examination of the test birds showed 3 brain (25% - 8% in each region, grade 1) lesions in treated birds and 1 (11%, grade 1) in the same control brain regions. Since these lesions were similar to those found in controls from previous studies, they were considered incidental.

The study supports a conclusion the disulfoton does not cause acute delayed neuropathy (OPIDN) in hens.
The study is acceptable for an acute delayed neurotoxicity study (OPPTS# 870.6100) in hens.

In an acute neurotoxicity study in Sprague-Dawley rats (10/sex/group), 97.8% disulfoton was administered by a single gavage dose of 0.25, 1.5, or 5.0 mg/kg in males and 0.25, 0.75, or 1.5 mg/kg in females. The NOAEL for neurotoxicity and cholinesterase inhibition was 0.25 mg/kg, based on muscle fasciculations in 4/10 females and plasma and RBC cholinesterase inhibition at the LOAELs of 0.75 mg/kg in females and 1.5 mg/kg in males. The incidence and type of clinical, behavioral, and neuromotor signs increased with dose. Females were clearly more sensitive. Neither brain weight nor neuropathology was affected by treatment (MRID 42755801). In a 90-day subchronic neurotoxicity study, 98.7-99.0% disulfoton was administered to Fisher 344 rats (1 2/sex/group) at dietary levels of 1, 4, or 16 ppm (0.063, 0.270, or 1.08 mg/kg/day in males and 0.071, 0.315, or 1.31 mg/kg/day in females). The systemic NOAEL was 1 ppm (0.063/0.071 mg/kg/day for M/F), based upon clinical signs consistent with cholinesterase inhibition (muscle fasciculations, urine staining, increased food consumption) in females at the LOAEL of 4 ppm (0.270/0.315 mg/kg/day in M/F). At 16 ppm (1.08/1.31 mg/kg/day in

M/F), treatment-related findings in both sexes also included increased reactivity, perianal staining, tremors, increased defecation, decreased forelimb grip strength, decreased motor and locomotor activity, decreased body weight gain, and corneal opacities. Cholinesterase inhibition (plasma, erythrocyte, and brain) was observed at all treatment levels (ChE NOAEL≤1 ppm; 0.063/0.071 mg/kg/day for M/F). Clearly females were again shown to be more sensitive. It was noted that clinical signs were persistent throughout this study. There were no treatment-related effects on brain weight. At the high-dose level, neuropathological lesions (nerve fiber degeneration) were observed in the optic nerve, and nerve fiber degeneration was also observed in the thoracic spinal cord. These findings, however, with similar neuropathy in control rats, the marginal increase in these lesions at the highest dose tested were not sufficiently different control lesions to indicate that treatment-related effect had occurred (MRID 42977401).

2. Developmental Toxicity

In a prenatal developmental toxicity study in Sprague-Dawley rats (25/group), 98.2% disulfoton was administered on gestation days 6-15 by gavage in polyethylene glycol 400 at dose levels of 0.1, 0.3, or 1.0 mg/kg/day. Cholinesterase activity was measured in dams (5/group) on gestation day 15. The maternal NOAEL was 0.1 mg/kg/day, and the maternal LOAEL was 0.3 mg/kg/day, based on 41% inhibition of plasma and RBC cholinesterase. There was no other evidence of maternal toxicity at any treatment level. The developmental NOAEL and LOAEL were established at 0.3 and 1.0 mg/kg/day, based on incomplete ossification of the intraparietals and sternebrae (MRID 00129458)

In a prenatal developmental toxicity study conducted in New Zealand white rabbits (15-22/group), 97.3% disulfoton was administered by gavage in corn oil (5 ml/kg) at doses of 0.3, 1.0, or 3.0 (reduced to 2.0, then 1.5) mg/kg/day on gestation days 6-18. The maternal NOAEL was 1.0 mg/kg/day; the maternal LOAEL (1.5 mg/kg/day) was based upon clinical signs of cholinesterase depression (tremors, unsteadiness/ incoordination, and increased respiration, occurring within 4 hours of dosing). In addition, there were a large number of mortalities at the high-dose level. There was no evidence of developmental toxicity (developmental NOAEL ≥1.5 mg/kg/day). Neither maternal nor fetal cholinesterase levels were measured (MRID 00147886).

3. Reproductive Toxicity:

In a two-generation reproduction study in Sprague-Dawley rats (25/sex/group), 97.8% disulfoton was administered at dietary concentrations of 1, 3, or 9 ppm (calculated effective doses of 0.81, 2.4, or 76.3 ppm; equivalent to 0.04, 0.12, or 0.36 mg/kg/day by test material consumption). The parental systemic NOAEL was 3 ppm (0.12 mg/kg/day). The parental systemic LOAEL was 9 ppm (0.36 mg/kg/day), based on decreased females mated and reduced body weight during gestation and lactation in P females.

The offspring NOAEL was 1 ppm (0.04 mg/kg/day), and the offspring LOAEL was 3 ppm (0.12 mg/kg/day), based on decreased brain cholinesterase activity in F1a weanling pups and on decreased F2b pup survival. Although adult cholinesterase was not measured, the 2-year chronic study indicates that cholinesterase inhibition was most likely occurring at 3 ppm with a NOAEL of 1 ppm; this was a conclusion of the 4/25/96 RfD PRC meeting (MRID 00157511).

In a another 2-generation reproduction study, disulfoton, technical (99% a.i.) was administered to 30 Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 0.5, 2.0 or 9.0 ppm (0, 0.025, 0.10 or 0.45 mg/kg/day by std. tables). Dosing was continuous for the P0 and F1 generation. Only one littering/animal/group was conducted. In this second 2-generation reproduction toxicity study with disulfoton, cholinesterase activity was measured in adults during pre-mating (at 8 weeks) and at termination and in pups at postnatal day 4 and day 21 in both generations. The major effects noted were cholinesterase inhibition and dams with no milk. In P0 males, plasma cholinesterase (PCHE) was significantly depressed and dose related pre-mating at 9.0 ppm (≥-34%) and at termination at 2.0 (≥-11%) and 9.0 ppm (-46%). In P0 females, plasma cholinesterase (PCHE) was significantly depressed pre-mating (≥-29%) and at termination (≥-52%) at ≥2.0 ppm. In P0 males and females erythrocyte cholinesterase (ECHE) was significantly depressed and dose related at ≥2.0 ppm (≥-38% & ≥-35% males and ≥-46% & ≥-80% females) a pre-mating and termination, respectively, but only in females at termination (≥-14%) at ≥0.5 ppm. In P0 males and females brain cholinesterase (BCHE) was significantly depressed and dose related at ≥2.0 ppm in males (≥-11%) and ≥-14% in females at ≥0.5 ppm. PCHE and ECHE depression in F1 males and females followed a similar nominal pattern to that in P0 males and females, except that the statistical significance varied within the F1 between two dose levels; sometimes the dose level showing statistical significance was higher and sometime lower of the two. In F1 males and females, BCHE was significantly depressed and dose related at ≥2.0 ppm in males (≥-14%) and in females (≥-50%). In F1 and F2 male and female pups at day 4 and/or day 21 of lactation, PCHE and ECHE were significantly depressed at 9.0 ppm. Values for PCHE and ECHE, respectively were at day 4 or day 21 in F1 male pups were (-24% & -47%) and for F1 female pups (-31% & -43%). Values for PCHE and ECHE, respectively, were at day 4 or day 21 in F2 male pups were (-46% & -53%) and for F2 female pups (-48% & -51%). In F1 and F2 male and female pups BCHE was significantly depressed at day 4 and day 21 at 9.0 ppm only (day 4 = -14% F1 males and -17% F1 females)(day 21 = -19% F1 males and -23% F1 females)(day 4 = -11% F2 males and -13% F2 females)(day 21 = -35% F2 males and -37% F2 females). Muscle fasciculation (one P0 female), tremors (15 P0 females, ten F1 females) and dams (seven F1 dams) with no milk were noted at 9.0 ppm. No treatment related organ weight changes or histopathology were noted in P0 or F1 males or females at any dose level. Clinical observations indicate that dams were not caring for their pups. Observed affects in pups in the 9.0 ppm group included 12 F1 (two dams) pups cold to the touch and three F1 (two dams) not being cared for and 63 F2 pups (seven dams) with no milk in their stomachs and 93 F2 weak pups (ten dams) from the affected dams. In addition, one P0 dam was salivating and gasping and did care for the litter and the litter died at 2.0 ppm. This effect at 2.0 ppm was considered test material related by the summary author of the 6(a)(2) submission (See summary 6(a)(2) report, MRID# 44440801; memorandum from David Anderson to PM 53, dated March 24, 1998, D242573), but ignored in the final report summary. Findings at necropsy were noted in F2 pups at 9.0 ppm that were expected in view of the maternal toxicity at this dose level. The report reasonably considered the pup deaths due to failure of maternal care, because of the weak and cold to the touch pups and failure of the pups to show milk in their stomachs. On careful examination of the report, this reviewer agrees with this conclusion. Thus, under these conditions, the effects in pups were caused by maternal toxicity and not the direct toxicity of disulfoton on pups.
Body weight change was lower than control values during gestation in P0 (-9%) and F1 (-15%) females. Body weights were significantly reduced at termination from control values in P0 (-6%) and F1 females

(-13%) and in F1 males (-8%). No other significant body weights or changes were noted. The P0 parental LOAELs were 0.5 ppm (0.025 mg/kg/day) based on brain cholinesterase activity depression in P0 females with tremors and muscle fasciculation at 9 ppm in females during gestation and lactation from both generations and with body weight decrements at 9.0 ppm, especially at termination. A NOAEL of 0.5 ppm (0.025 mg/kg/day) was seen in F1 parents. F1 and F2 pup (4 days and 21 days old) cholinesterase activity, including brain cholinesterase activity was depressed only at 9.0 ppm (0.45 mg/kg/day) with 2.0 ppm (0.10 mg/kg/day) being the NOAEL. The F1 pup NOAEL/LOAEL were 2.0/9.0 ppm (0.10/0.45 mg/kg/day) based on treatment related pup deaths and pup weight decrements at 9.0 ppm, probably from inadequate maternal care (MRID# 44440801).

4. Additional Information from the Literature

This summary is provided to develop a comprehensive picture of disulfoton toxicity. The data have not been reviewed in depth, and no statement is made regarding the accuracy or quality of the data or reports.

In a 1988 study by McDonald et al., disulfoton was administered by daily i.p. injection at 2 mg/kg/day to male Long-Evans rats for 14 days. In treated rats, muscarinic receptor binding was decreased and spacial memory was decreased in a T-maze alternation task.

5. Determination of Suseptibility

There is no quantitative or qualitative evidence of increased susceptibility of fetuses following *in utero* exposure to rats and rabbits and during pre/post natal exposure to rats. In these studies, toxicity to the fetus or pups occurred only at higher dose levels than to the dams (mothers) or parents.

6. Recommendation for Developmental Neurotoxicity Study

A developmental neurotoxicity study with disulfoton is required by the Data Call-In Notice (September 10, 1999) for select organophosphates.

7. Determination of the FQPA Safety Factor:

The FQPA Safety Factor Committee met on January 24, 2000 to re-evaluate the hazard and exposure data for disulfoton, and recommended that the FQPA Safety Factor (as required by Food Quality Protection Act of August 3, 1996) be removed (1x) in assessing the risk posed by this chemical. The FQPA safety factor recommendation in this report supercedes that previously reported for disulfoton in the *FQPA SAFETY FACTOR RECOMMENDATIONS FOR THE ORGANOPHOSPHATES* dated August 6, 1998.

6. DATA GAPS

Developmental neurotoxicity study as required by the Data Call-In Notice (September 10, 1999)

7. HAZARD CHARACTERIZATION

Cholinesterase inhibition (plasma, erythrocyte and/or brain) is seen at the lowest dose levels tested in rats, mice, rabbits and dogs. All of the endpoints are based on good dose related responses in cholinesterase inhibition. Many of the studies show clinical signs at higher dose levels. Females appear to be more sensitive to cholinesterase inhibition in most studies.

The organophosphates have a common mode of action in that they decrease erythrocyte and/or brain cholinesterase in animals and humans. Plasma cholinesterase inhibition is a surrogate for possible muscle and brain cholinesterase inhibition. Neuropathy may result from higher exposures to these inhibitors. The rabbit 21-day dermal studies did not show as consistent cholinesterase inhibition with time as other studies showed, although the 3-day dermal rat study showed a time dependence between day 2 and day 4. The results were somewhat dependent on whether concurrent controls were used or the values for the individual animals at the beginning of the study were used for comparison.

Cholinesterase inhibition occurred at the LOAEL in rats, mice, rabbits and dogs. Therefore the effects are very uniform across species. The female of the species appears to be more sensitive than the male and the cholinesterase inhibition occurs at slightly different dose levels across the species. The cholinesterase inhibition appears to be slightly greater in the female than the male in most studies.

Adequate developmental toxicity and reproductive toxicity studies show adult toxicity occurs at lower dose levels than toxicity to the fetus or offspring. There is no evidence to support increased susceptibility following pre natal exposure to rat or rabbit fetuses or pre/post natal exposure to rats. In these studies, toxicity to the fetus or pups occurred only at higher dose levels than to the adults (dams or parents). Thus, there is no evidence of increased susceptibility to the fetus or to offspring.

The following literature summary is provided to develop a comprehensive picture of disulfoton toxicity. The data have not been reviewed in depth, and no statement is made regarding the accuracy or quality of the data or reports. In a 1988 study by McDonald et al., disulfoton was administered by daily i.p. injection at 2 mg/kg/day to male Long-Evans rats for 14 days. In treated rats, muscarinic receptor binding was decreased and spacial memory was decreased in a T-maze alternation task. These effects occurred in the presence of -75% brain ChEI therefore the effects may not be relevant at the NOAEL for brain ChEI.

8. ACUTE TOXICITY ENDPOINTS:

Acute Toxicity of disulfoton, technical

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral	Acc# 072293	LD_{50} = M: 6.2 mg/kg; F:1.9 mg/kg	I
81-2	Acute Dermal	Acc# 07793	LD_{50} = M: 15.9 mg/kg; F: 3.6 mg/kg	I
81-3	Acute Inhalation	Acc# 258569	LC_{50} = M: 0.06 mg/L; F: 0.89 mg/L	I
81-4	Primary Eye Irritation	None	Data requirement waived.	N/A
81-5	Primary Skin Irritation	None	Data requirement waived.	N/A
81-6	Dermal Sensitization	None	Data requirement waived.	N/A
81-7	Acute Delayed Neurotoxicity	00129384	Equivocal	
81-8	Acute Neurotoxicity	42755801	Reversible neurotoxic signs consistent with the cholinesterase inhibition 1.5 mg/kg in females and 5.0 mg/kg in males	N/A

9. SUMMARY OF TOXICOLOGY ENDPOINTS

The doses and toxicological endpoints selected for various exposure scenarios are summarized in the table below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL = 025	Plasma and RBC cholinesterase inhibition and clinical signs of toxicity.	Acute Neurotoxicity-Rat
	UF=100	**Acute RfD = 0.0025 mg/kg**	
Chronic Dietary	NOAEL = 0.013	Plasma, RBC, brain and ocular cholinesterase inhibition	Chronic Toxicity-Dog
	UF=100	**Chronic RfD = 0.00013 mg/kg/day**	
Dermal, Short-Term	Dermal NOAEL = 0.5	Plasma and brain cholinesterase inhibition	3-Dermal Toxicity-Rat
Dermal, Intermediate-Term	Oral NOAEL=0.03[a]	Plasma, RBC and brain cholinesterase inhibition	6-Month Cholinesterase-Rat
Dermal, Long-Term	Oral NOAEL=0.013 [a]	Plasma, RBC, brain and ocular cholinesterase inhibition	Chronic Toxicity-Dog
Inhalation, Short, Intermediate and Long-Term	Inhalation NOAEL= 0.00016 mg/L or 0.045 mg/kg/day	Plasma, RBC and brain cholinesterase inhibition	90-Day Inhalation Toxicity- Rat

[a] Use 36% dermal absorption factor for route-to-route extrapolation.